

Effects of *Bacillus subtilis* Supplementation in Diets with Different Zinc Levels on Growth Performance, Slaughter Performance, Meat Quality, and Nutrient Utilization in Meat Geese: A Post-print

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Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary *Bacillus subtilis* supplementation at different zinc levels on growth performance, slaughter performance, meat quality, and nutrient utilization in meat geese, and to determine the appropriate dietary zinc supplementation level under *Bacillus subtilis* supplementation conditions. A total of 360 five-week-old Wulong geese with similar body condition were selected and randomly divided into 6 groups, with 6 replicates per group and 10 geese per replicate. The control group received the basal diet supplemented with 80 mg/kg zinc without *Bacillus subtilis*; Groups I-V were experimental groups, receiving the basal diet supplemented with 0, 20, 40, 60, and 80 mg/kg zinc, respectively, with *Bacillus subtilis* supplementation level at 250 mg/kg for all groups. The experimental period lasted 11 weeks. The results showed: 1) The body weight and average daily gain of geese in Group III were significantly higher than those in Groups I and V ($P < 0.05$), and the feed-to-gain ratio of geese in Group IV was significantly lower than that in Groups I and V ($P < 0.05$). The average daily gain was maximized at a dietary zinc supplementation level of 47.5 mg/kg, and the feed-to-gain ratio was minimized at a dietary zinc supplementation level of 15.0 mg/kg. 2) The dressing percentage of geese in Groups III, IV, and V was significantly higher than that in Groups I and II ($P < 0.05$). 3) The muscle water loss rate of geese in Groups II and III was significantly lower than that in Groups IV and V ($P < 0.05$). 4) The crude protein utilization rate of geese in Groups II, III, and IV was significantly higher than that in Group I ($P < 0.05$); the crude fat utilization rate of geese in Group IV was extremely significantly higher than that in Group II ($P < 0.01$) and significantly higher than that in Group V ($P < 0.05$); the

crude fiber utilization rate of geese in Groups II, III, and IV was significantly higher than that in Groups I and V ($P < 0.05$); the zinc utilization rate of geese in Groups I, II, and III was significantly lower than that in Groups IV and V ($P < 0.05$). 5) The excreted nitrogen of geese in Groups II and III was significantly lower than that in Group I ($P < 0.05$), and the nitrogen utilization rate of geese in Groups IV and V was significantly higher than that in Groups I, II, and III ($P < 0.05$). 6) Through paired t-test analysis, compared with the control group (without *Bacillus subtilis* supplementation), the body weight, dressing percentage, breast muscle percentage, abdominal fat percentage, muscle redness value, as well as crude protein, neutral detergent fiber, and nitrogen utilization rates of geese in Group V (with *Bacillus subtilis* supplementation) were all significantly increased ($P < 0.05$). Thus, dietary supplementation of *Bacillus subtilis* and zinc can both improve growth performance, slaughter performance, and the utilization rates of crude protein, crude fat, crude fiber, and zinc, while reducing zinc and nitrogen emissions. Under the condition of dietary supplementation with 250 mg/kg *Bacillus subtilis*, the appropriate zinc supplementation level is 15.0-47.5 mg/kg.

Full Text

Effects of *Bacillus subtilis* Supplementation in Different Zinc Supplemental Level Diets on Growth Performance, Slaughter Performance, Meat Quality and Nutrient Utilization of Meat Geese

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Abstract: This experiment was conducted to investigate the effects of *Bacillus subtilis* supplementation in diets with varying zinc levels on growth performance, slaughter performance, meat quality, and nutrient utilization in meat geese, and to determine the optimal dietary zinc supplementation level when *Bacillus subtilis* is added.

A total of 360 five-week-old Wulong geese with similar body condition were randomly allocated into 6 groups, each consisting of 6 replicates with 10 geese per replicate. The control group received a basal diet supplemented with 80 mg/kg zinc without *Bacillus subtilis*, while experimental groups through received basal diets supplemented with 0, 20, 40, 60, and 80 mg/kg zinc, respectively, all containing 250 mg/kg *Bacillus subtilis*. The trial lasted for 11 weeks. The results showed: (1) Body weight and average daily gain (ADG) in group were significantly higher than those in groups and ($P < 0.05$), while feed-to-gain ratio (F/G) in group was significantly lower than in groups and ($P < 0.05$).

Maximum ADG was achieved at 47.5 mg/kg zinc supplementation, and the lowest F/G occurred at 15.0 mg/kg zinc. (2) Dressed percentage in groups , , and was significantly higher than in groups and ($P < 0.05$). (3) Muscle water loss rate in groups and was significantly lower than in groups and ($P < 0.05$). (4) Crude protein utilization in groups , , and was significantly higher than in group ($P < 0.05$). Ether extract utilization in group was significantly higher than in group ($P < 0.01$) and group ($P < 0.05$). Crude fiber utilization in groups , , and was significantly higher than in groups and ($P < 0.05$). Zinc utilization in groups , , and was significantly lower than in groups and ($P < 0.05$). (5) Nitrogen excretion in groups and was significantly lower than in group ($P < 0.05$), while nitrogen utilization in groups and was significantly higher than in groups , , and ($P < 0.05$). (6) Paired t-test analysis revealed that compared with the control group (without *Bacillus subtilis*), group (with *Bacillus subtilis*) showed significant increases in body weight, dressed percentage, breast muscle percentage, abdominal fat percentage, meat redness value, and utilization of crude protein, neutral detergent fiber, and nitrogen ($P < 0.05$). In conclusion, dietary supplementation with *Bacillus subtilis* and zinc can improve growth performance, slaughter performance, and utilization of crude protein, ether extract, crude fiber, and zinc, while reducing zinc and nitrogen emissions. Under conditions of 250 mg/kg *Bacillus subtilis* supplementation, the appropriate dietary zinc level is 15.0–47.5 mg/kg.

Keywords: *Bacillus subtilis*; zinc; geese; growth performance; slaughter performance; meat quality; nutrient utilization

Zinc is an essential trace element in animal growth that plays a vital role in development and metabolism, earning it the designation “life element.” *Bacillus subtilis* exhibits adsorptive properties for divalent metal ions, binding them in the intestinal tract to form chelates with amino acids and other compounds produced by the bacteria, thereby facilitating absorption or excretion and promoting healthy growth. With the rapid development of animal production in China, environmental pollution from the livestock industry has become increasingly severe. Heavy metal pollution is particularly concerning due to its cumulative, irreversible, and long-term consequences, making it a persistent research focus in environmental and ecological sciences both domestically and internationally. Unabsorbed zinc excreted by animals represents a significant source of heavy metal contamination. Therefore, research on *Bacillus subtilis* supplementation in low-zinc diets holds important practical significance.

Numerous studies have demonstrated that *Lactobacillus* produces antibacterial substances that inhibit putrefactive products from intestinal microflora, improving gut environment and thereby enhancing health and longevity. As a probiotic preparation, *Bacillus subtilis* effectively improves intestinal microflora composition and promotes nutrient absorption. López et al. reported that *Bacillus* strains possess strong metal adsorption capacity because metal ions can be im-

mobilized through interactions with anions on the bacterial cell surface. Jayaraman et al. found that *Bacillus subtilis* PB6 can inhibit necrotic enteritis caused by *Clostridium perfringens* in broiler chickens, improving intestinal health. Zinc is one of the most functionally diverse trace elements in animals, essential for poultry growth, feather development, and skeletal formation. It participates in the metabolism of the three major nutrients, nucleic acids, vitamins, and other trace elements, and is required for the activity of nearly 300 enzymes across six enzyme systems, making it indispensable for physiological functions including bone development, reproduction, immunity, and blood coagulation. While numerous studies have investigated the individual functions of zinc and *Bacillus subtilis*, research on their synergistic effects remains limited, particularly regarding the potential of *Bacillus subtilis* to reduce required zinc supplementation levels. Therefore, this study used 5- to 15-week-old Wulong geese as an animal model to investigate the effects of *Bacillus subtilis* supplementation in diets with varying zinc levels on growth performance, slaughter performance, meat quality, and nutrient utilization, aiming to determine the optimal zinc supplementation level when *Bacillus subtilis* is added and provide technical support for reducing trace mineral usage.

1.1 Experimental Animals and Design

Three hundred sixty 5-week-old Wulong geese with similar body condition were randomly divided into 6 groups using a random allocation numbering method, with 6 replicates per group and 10 geese per replicate (equal numbers of males and females). The experimental design is shown in Table 1 . The control group received a basal diet supplemented with 80 mg/kg zinc without *Bacillus subtilis*. Experimental groups through received basal diets supplemented with 0, 20, 40, 60, and 80 mg/kg zinc, respectively, all containing 250 mg/kg *Bacillus subtilis*. The trial lasted for 11 weeks. Experimental geese were provided by the breeding base of the Institute of Quality Waterfowl at Qingdao Agricultural University. The zinc source was zinc sulfate heptahydrate (98% active ingredient) purchased from Zhejiang Xinweipu Additive Co., Ltd., and *Bacillus subtilis* was purchased from Jiangsu Yuanfang Zhonghui Biotechnology Co., Ltd.

1.2 Experimental Diets

The basal diet was formulated using corn and soybean meal as primary ingredients, with nutrient levels designed according to NRC (1994) recommendations for geese. The composition and nutrient levels of the basal diet are presented in Table 2 . The zinc content in the basal diet was measured as 22.65 mg/kg using inductively coupled plasma optical emission spectrometry.

1.3 Management Practices

Before the feeding trial, the goose house and equipment were washed and disinfected with caustic soda solution spray, then fumigated with formaldehyde

and potassium permanganate (28 mL formaldehyde and 14 g potassium permanganate per m³) with doors and windows sealed for 24 hours. The trial began one week later. Geese were raised on net beds with ad libitum access to feed and water throughout the experimental period. The house was thoroughly washed and strictly disinfected before the trial and disinfected twice weekly during the trial. Temperature and humidity were measured using a dry-wet bulb thermometer three times daily at 08:00, 14:00, and 20:00.

1.4 Measurement Indicators

1.4.1 Growth Performance At the end of week 15, geese were fasted and weighed by replicate to calculate average daily gain (ADG) from 5 to 15 weeks. Daily feed consumption was recorded to determine average daily feed intake (ADFI). Mortality was recorded daily, and these data were used to calculate feed-to-gain ratio (F/G) [6].

1.4.2 Slaughter Performance At the end of week 15, 12 geese from each group (2 per replicate, equal numbers of males and females) with body weight close to the group mean were selected (total of 72 geese). After 12 hours of fasting, live weight was recorded and geese were slaughtered by jugular vein exsanguination. Following the “Poultry Performance Terminology and Measurement Methods” (NY/T 823-2004), wet plucking was performed and moisture was drained before recording carcass weight. Dressing percentage, half-eviscerated percentage, eviscerated percentage, breast muscle percentage, leg muscle percentage, and abdominal fat percentage were calculated from measurements of carcass weight, half-eviscerated weight, eviscerated weight, abdominal fat weight, breast muscle weight, and leg muscle weight.

1.4.3 Meat Quality At slaughter, meat samples were taken from the central portion of the pectoralis major muscle using a scalpel. After color development at 25 °C for 30 minutes, meat quality parameters were measured: meat color using a Japanese automatic colorimeter, shear force using a texture analyzer (TA-XT PLUS), pH using a portable pH meter (HANHA-HI9025), and water loss rate using a pressure meter.

1.4.4 Nutrient Utilization At the end of week 15, 6 geese from each group (equal numbers of males and females) were randomly selected and moved into metabolic cages (Patent No.: 200720177297) for a 4-day adaptation period. After 1 day of fasting, the formal 3-day trial began with ad libitum water and 120 g of diet provided daily. Excreta were collected continuously for 4 days using the total collection method, with nitrogen fixed by hydrochloric acid and mixed samples taken.

Feed samples were ground to 40 mm and stored at low temperature after drying. Excreta samples were dried in an oven at 65–75 °C, then ground using a small universal grinder. Metabolizable energy (ME) was determined by bomb

calorimetry, crude protein (CP) by semi-automatic Kjeldahl nitrogen analyzer, zinc content by inductively coupled plasma optical emission spectrometry (ICP), crude fiber (CF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) by ANKOM220 Fiber Analyzer (ANKOM Company), and ether extract (EE) by ether extraction method.

1.5 Data Processing and Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 22.0 to examine the effects of different dietary zinc levels with *Bacillus subtilis* supplementation on various parameters, with linear and quadratic relationships analyzed for dietary zinc levels. To evaluate the effect of *Bacillus subtilis*, paired t-tests were conducted between the control group and group (both with 80 mg/kg zinc, but without and with 250 mg/kg *Bacillus subtilis*, respectively). Data are presented as least squares means with standard error of the mean (SEM). $P < 0.05$ indicated significant difference, and $P < 0.01$ indicated highly significant difference.

2.1 Effects on Growth Performance

As shown in Table 3, dietary zinc level had highly significant effects on body weight, ADG, and F/G ($P < 0.01$). Body weight and ADG were highest in group, significantly exceeding those in groups and ($P < 0.05$). F/G was lowest in group, significantly lower than in groups and ($P < 0.05$). No significant differences in ADFI were observed among groups ($P > 0.05$).

Paired t-test analysis (Table 4) revealed that compared with the control group (without *Bacillus subtilis*), group (with *Bacillus subtilis*) showed significantly increased body weight ($P < 0.05$) and reduced F/G ($P > 0.05$). These results indicate that *Bacillus subtilis* supplementation effectively improves growth performance in meat geese at the same dietary zinc level.

Quadratic regression relationships existed between body weight, ADG, F/G and dietary zinc level ($P < 0.05$). Quadratic curve fitting for ADG (Y1) and F/G (Y2) against dietary zinc level (X) in groups - yielded the following equations:

$$Y1 = -0.001X^2 + 0.095X + 30.537 (R^2 = 0.636, PQ = 0.000)$$

$$Y2 = 0.001X^2 - 0.030X + 6.387 (R^2 = 0.630, PQ = 0.000)$$

These equations indicate maximum ADG at 47.5 mg/kg zinc supplementation and minimum F/G at 15.0 mg/kg zinc. From a comprehensive benefit perspective, the recommended dietary zinc level is 15.0-47.5 mg/kg when *Bacillus subtilis* is supplemented at 250 mg/kg.

2.2 Effects on Slaughter Performance

Table 5 shows that dietary zinc level had a highly significant effect on dressed percentage ($P < 0.01$). Groups, , and exhibited significantly higher dressed percentages than groups and ($P < 0.05$), with group showing the highest

value. No significant differences were observed among groups for half-eviscerated percentage, eviscerated percentage, breast muscle percentage, leg muscle percentage, or abdominal fat percentage ($P > 0.05$), though group had lower values than the other four experimental groups.

Paired t-test analysis (Table 6) indicated that group had significantly higher dressed percentage, breast muscle percentage, and abdominal fat percentage than the control group ($P < 0.05$), while half-eviscerated and eviscerated percentages were higher but not significant ($P > 0.05$). These results demonstrate that the combination of *Bacillus subtilis* and zinc promotes slaughter performance in meat geese.

Quadratic curve fitting for dressed percentage (Y3) against dietary zinc level (X) in groups - produced the equation:

$$Y3 = 76.797 + 0.508X - 0.006X^2 (R^2 = 0.618, PQ = 0.000)$$

This equation indicates maximum dressed percentage at 42.33 mg/kg zinc. From a comprehensive benefit perspective, the optimal dietary zinc level for maximizing dressed percentage is 42.33 mg/kg when *Bacillus subtilis* is supplemented at 250 mg/kg.

2.3 Effects on Meat Quality

Table 7 reveals that dietary zinc level had a highly significant effect on muscle water loss rate ($P < 0.01$). Groups and showed significantly lower water loss rates than groups and ($P < 0.05$), with group having the lowest value. No significant differences were observed among groups for meat color, shear force, or pH ($P > 0.05$). These results suggest that dietary zinc levels exceeding 40 mg/kg do not significantly affect meat quality physicochemical properties.

Paired t-test analysis (Table 8) showed that group had significantly higher meat redness value than the control group ($P < 0.05$), with no significant differences in lightness, yellowness, shear force, pH, or water loss rate ($P > 0.05$).

2.4 Effects on Nutrient and Energy Utilization

Table 9 demonstrates that dietary zinc level had significant effects on crude protein and ether extract utilization ($P < 0.05$) and highly significant effects on crude fiber and zinc utilization ($P < 0.01$). Crude protein utilization in groups , , and was significantly higher than in group ($P < 0.05$). Ether extract utilization in group was significantly higher than in group ($P < 0.01$) and group ($P < 0.05$). Crude fiber utilization in groups , , and was significantly higher than in groups and ($P < 0.05$). Zinc utilization in groups , , and was significantly lower than in groups and ($P < 0.05$). No significant differences were observed among groups for neutral detergent fiber or acid detergent fiber utilization ($P > 0.05$). Group showed the highest crude protein, ether extract, and crude fiber utilization.

Paired t-test analysis (Table 10) revealed that group had significantly higher crude protein and neutral detergent fiber utilization than the control group ($P < 0.05$), with no significant differences in ether extract, crude fiber, acid detergent fiber, or zinc utilization ($P > 0.05$).

Table 11 shows that dietary zinc level had significant effects on nitrogen excretion and utilization ($P < 0.05$). Nitrogen excretion in groups and was significantly lower than in group ($P < 0.05$) but not significantly different from groups and ($P > 0.05$). Nitrogen utilization in groups and was significantly higher than in groups , , and ($P < 0.05$).

Paired t-test analysis (Table 12) indicated that group had significantly higher nitrogen utilization than the control group ($P < 0.05$), with no significant differences in nitrogen intake, excretion, or deposition ($P > 0.05$).

Tables 13 and 14 show no significant differences among groups for gross energy intake, fecal energy, endogenous energy, apparent metabolizable energy, true metabolizable energy, or energy utilization ($P > 0.05$). Paired t-test analysis also revealed no significant differences between the control group and group for any energy utilization parameters ($P > 0.05$).

3.1 Effects on Growth Performance

Growth performance reflects animal development, and the performance of young animals during their most vigorous growth phase directly affects subsequent development. *Bacillus subtilis* promotes nutrient absorption, improves feed conversion efficiency, prevents disease, and enhances animal growth. Studies have shown that *Bacillus* supplementation can replace antibiotics in broiler diets to promote growth and improve feed efficiency. Ma reported that 80 mg/kg zinc supplementation improved growth performance in broilers during the late growth phase. Su et al. found that increasing dietary zinc levels promoted growth performance in ducklings, with an optimal level of 51.8 mg/kg. Hooge et al. confirmed the growth-promoting effects of *Bacillus subtilis* in broilers and meat geese. Our results showed that *Bacillus subtilis* supplementation increased body weight compared with the control group at the same zinc level. Among the experimental groups, group showed poorer growth performance and exhibited zinc deficiency symptoms. Notably, at 250 mg/kg *Bacillus subtilis* supplementation, F/G decreased by 13.17% at 15.0 mg/kg zinc compared with the control, while ADG increased by 10.30% at 47.5 mg/kg zinc. These findings demonstrate that the synergistic effect of *Bacillus subtilis* and zinc significantly improves growth performance while effectively reducing required zinc levels.

3.2 Effects on Slaughter Performance

Slaughter performance is a key indicator for evaluating poultry breed quality, feeding management, and processing efficiency, directly reflecting body composition and edible portion proportions. Some studies have found no significant

effect of dietary zinc level on slaughter performance. Tang et al. reported that zinc bacitracin supplementation significantly improved growth and slaughter performance in Peking ducks compared with β -glucan and control groups. Dietary supplementation with 500 mg/kg *Bacillus subtilis* significantly increased dressed percentage in 35-day-old broilers without affecting other slaughter parameters, while 1% *Bacillus subtilis* improved breast muscle quality in Silkie chickens. Our results showed that *Bacillus subtilis* supplementation significantly increased dressed percentage compared with the control, and varying zinc levels tended to improve slaughter performance under *Bacillus* supplementation. This indicates that the combination of *Bacillus subtilis* and zinc promotes slaughter performance while effectively reducing dietary zinc requirements.

3.3 Effects on Meat Quality

Research on the relationship between *Bacillus subtilis* and poultry carcass quality is limited. Liu found that zinc supplementation in broiler diets promoted growth, improved carcass performance, and enhanced meat quality. Ren et al. reported that lower shear force in breast muscle indicates better meat quality. Our results showed that *Bacillus subtilis* supplementation alone did not significantly affect meat quality, but dietary zinc level significantly influenced muscle water loss rate. Research on the effects of zinc and *Bacillus subtilis* on meat goose quality remains scarce, and the underlying mechanisms require further investigation.

3.4 Effects on Nutrient and Energy Utilization

Animal excreta are a major source of environmental pollution, with excessive mineral element excretion, particularly trace elements, being a primary contributor. Trace element excretion is closely related to both supplementation levels and bioavailability. Nutrient utilization directly reflects metabolic rate and growth performance. Neto et al. found that chelated zinc improved lysine utilization efficiency in laying hens. *Bacillus subtilis* possesses strong protease, lipase, and amylase activities, produces antibiotics, and exhibits strong oxygen-competing capacity in the intestinal tract, all of which promote digestion, improve feed conversion, and enhance growth. Previous research showed that 250 mg/kg *Bacillus subtilis* combined with 8 mg/kg copper significantly improved crude protein, crude fiber, copper, and nitrogen utilization while reducing fecal nitrogen and markedly increasing acid detergent fiber utilization in geese. Our results demonstrated that at 250 mg/kg *Bacillus subtilis* supplementation, dietary zinc level significantly or highly significantly affected crude protein, ether extract, crude fiber, and zinc utilization, while *Bacillus*-supplemented groups showed significantly higher nitrogen utilization than the control. These findings indicate that *Bacillus subtilis* promotes zinc absorption and utilization, improves nutrient utilization, allows for reduced zinc supplementation, decreases zinc and nitrogen emissions, and benefits environmental protection, while also demonstrating synergistic effects with zinc.

4 Conclusion

Dietary supplementation with *Bacillus subtilis* and zinc can improve growth performance, slaughter performance, and utilization of crude protein, ether extract, crude fiber, and zinc, while reducing zinc and nitrogen emissions. Under conditions of 250 mg/kg *Bacillus subtilis* supplementation, the appropriate dietary zinc level is 15.0–47.5 mg/kg.

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